Biomarker use in tailored combat casualty care

Modern war wounds are complex and primarily involve extremities. They require multiple operative interventions to achieve wound closure and begin rehabilitation. Current assessment of the suitability of surgical wound closure is based upon subjective methods coupled with a semiquantitative determination of the wound bacterial burden. Measurement of the systemic and local response to injury using inflammatory biomarkers may allow for accelerated wound closure and treatment of other combat-related morbidity. This article presents the introduction of personalized medicine into combat casualty care.

KEYWORDS: biomarker combat inflammation personalized medicine trauma war surgery wound

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Biomarkers

Modern day war surgery is dominated by the need to manage open wounds that require delayed closure and consume significant resources in the efforts toward appropriate closure. The ability to subjectively predict which wounds will fail is historically poor. Some wounds that appear destined for proper healing after debridement and tension-free closure instead continue on to dehiscence and require a return to surgical care. Similarly, wounds that appear grossly abnormal, but are biologically capable of healing, endure unnecessary surgical procedures with their corresponding risks and costs. Currently, the decision to close a wound is based on the surgeon's subjective evaluation of the wound, including appearance of granulation tissue, assessment of vascularization and an absence of gross infection. These subjective measurements of wounds are notoriously poor. Ideally, one would be able to monitor a patient's, or even an individual wound's, progression through the phases of wound healing using biomarkers as objective measures predictive of wound outcome to guide the timing of successful surgical closure. The role of biomarkers in wound healing has primarily been in the systemic evaluation of a patient's overall clinical status. Serum biomarkers have been described for wound healing in chronically malnourished elderly or chronically debilitated patients with modest success and have been found inappropriate or ineffective in traumatic acute injuries, as seen with combat casualties. For such patients with complex trauma, appropriate biomarkers of local (via wound effluent and wound tissue biopsies) and systemic (via serum) wound healing are on the horizon.

In order to understand the current status of biomarkers in war-associated injuries, it is imperative that one understands the history of the types of wounds experienced during various wars, the early work on biomarkers and, finally, the basic components of local wound healing.

Wartime injuries

The number of wartime casualties dying from their wounds has steadily declined, from 8% in World War I (WWI), 4.5% in World War II (WWII), 2.5% in Korea, 3.6% in Vietnam and 2.1% in Desert Storm [1]. The drastic decline in lethal wounds has been traded for a significant increase in the extremity wounds seen by war surgeons. Extremity wounds account for the majority of the injuries (65%) followed by head and neck (15%), thorax (10%), and abdomen (7%) [2]. Wartime blast injuries can be classified as [3,4]:

- Primary: injury caused by blast wave;
- Secondary: ballistic trauma resulting from fragmentation of the weapon or environment;
- Tertiary: resulting from the displacement of the victim or environmental structures;
- Quaternary: burns, toxins and radiologic contamination.

In Iraq and Afghanistan, the majority of current war wounds are caused by improvised explosive devices that predominately induce secondary and tertiary blast injuries, which contribute to extremity wounds being the most common combat injury in the modern wartime era [5].

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High-energy penetrating wounds, an example of secondary injuries, cause extensive multisystem trauma with concomitant gross bacterial contamination. Up to 75% of these injuries are colonized or infected with environmental bacteria when the patients arrive at tertiary care facilities [6]. The level of bacterial contamination significantly alters the ability of wounds to heal or qualify for flap coverage.

The management of war-associated injuries has been addressed throughout medical history. One of the earliest patterns comes from the Greek literature describing a penetrating wound, where wound management consisted of removing the arrow, cleansing the wound with water, and applying analgesic and herbal medicines. John Hunter, a distinguished surgeon in the 18th century, who served as the British Surgeon General, advocated minimal surgical debridement of infected wounds [7]. During the US Civil War, the concept of removal of necrotic and infected tissue was addressed through early amputation. Given the lack of antibiotics and the strong association of infection with mortality, surgeons understood the risk associated with an infected limb. In WWI, the initial understanding of infection and wound healing began to surface when primary repair was attempted in 224 gunshot wounds at a casualty clearing station [8]. A portion of these wounds were culture negative and healed. In the remaining infected wounds, a variety of bacteria were identified. Although a few of those culture-positive wounds were able to undergo primary wound closure, the presence of hemolytic streptococcus was associated with failed closure in 95% of them. During WWII, and with the advent of antimicrobials, the literature began to focus on wound bacteriology, susceptibility of various microbes to the available antimicrobial agents, and the critical nature of time. These concepts of the interplay of infection and wound healing continued to progress and direct primary wound-closure decisions.

Quantitative bacterial counts as the initial biomarker of wound healing

Causes of pathologic or clinical infection include exogenous and endogenous factors [9]. One such contributing factor is simply the degree of bacterial colonization. Traumatic wounds are notoriously contaminated, especially in the setting of military trauma. For example, over 70% of combat wounds from Vietnam were clinically infected, in that they contained bacterial counts greater than 10^5 colony-forming units per gram of tissue (CFU/g), where greater than 10^5 CFU/g is considered clinical infection [10]. A second key factor is the time from injury to treatment [11]. When the mean time from injury to treatment is 2.2 h, wounds had bacterial counts of 10^2 CFU/g whereas a time of 3 h led to higher bacterial counts of 10^2–10^5 CFU/g, and a time of 5.17 h contained greater than 10^5 CFU/g. Therefore, it has been shown that the greater the amount of time from injury to treatment, the more bacterial colonization occurs and, subsequently, the greater the risk of imminent wound infection.

A direct relationship between bacterial counts and wound healing has been established for many years [12]. The direct association was described in pressure ulcers where healing occurred only when the bacterial counts were less than 10^6/ml [12]. The association has also been demonstrated in many wound models. In a 1967 study of 50 granulating wounds, graft survival was 94% when bacterial counts were less than 10^4 CFU/g and 19% when bacterial counts were greater than 10^5 CFU/g [13]. Similar results were found in a 1968 delayed wound-closure study where topical antibiotics were used for controlling bacteria in the wound [14]. In a review of the bacterial counts of the 40 wounds, 28 of the 30 wounds containing fewer than 10^5 CFU/g had uncomplicated healing during delayed closure; however, none of the wounds that had greater than 10^5 CFU/g experienced successful closure [15]. Following these studies, in a prospective evaluation of wound closure with quantitative bacteriology, 89 out of 93 wounds having less than 10^5 CFU/g progressed to rapid uncomplicated healing [16].

These studies led to the understanding of the effect of wound contamination on wound healing and presented the quantitation of bacterial CFU/g as a predictor of a wound's ability to heal. However, we are now learning that other factors may also influence wound healing. Inflammation, the body's natural response to injury, is caused by the signaling of cytokines and chemokines. These inflammatory mediators can also be measured as a predictor of wound healing. Therefore, evaluation of the natural response to injury, in addition to bacterial contamination, would potentially further refine the understanding of successful wound healing.

Wound healing

Wound healing can be described as occurring in four phases: hemostasis, inflammation, proliferation and maturation [17]. During hemostasis, platelet-mediated activation of the intrinsic

clotting cascade and vasoconstriction result in clot formation. The clot forms the support for infiltrating inflammatory cells as the platelets release inflammatory mediators, including cytokines, chemokines and growth factors. Over the ensuing inflammatory phase, neutrophils, macrophages and fibroblasts migrate into the tissue guided by various chemoattractants and cytokines. Chemoattractants that are important to the inflammatory phase include CXC and CC chemokines, and associated proinflammatory cytokines include IL-1, TNF-α, TGF-β, platelet factor 4 and leukotriene B4. The orchestration of these factors leads to the release of nitric oxide, oxygen free radicals, serine proteases and matrix metalloproteinases (MMPs) that destroy bacteria, and clear damaged extracellular matrix molecules and inflammatory debris [18]. In the proliferative phase, the wound begins epithelization, angiogenesis and matrix formation. Finally, in maturation, the wound undergoes contracture and migration of epithelial cells from the periphery. The wound matrix undergoes a change to become a more organized structure of collagen, proteoglycan and fibronectin. The understanding and evaluation of these factors and their substrates, as well as the systemic response to injury, will lend them utility as potential biomarkers in the prediction of wound healing in wartime-associated injuries.

Current efforts to predict war wound outcome

In our research program, our approach to combat casualty care is to take our bedside understanding of the wound healing process into the laboratory where we can quantitate the biomolecular mediators of the aforementioned phases. We then return to the bedside with that knowledge as a decision-supportive diagnostic tool for use by the surgical team for directed patient care in the spirit of translational medicine [19]. Our initial efforts have focused on the identification of biomarkers for timing surgical wound closure to ensure healing and avoidance of dehiscence, stratifying patient risk of heterotopic ossification and identifying detrimental microbial colonization. For the purpose of this article, we will focus on the use of biomarkers of wound healing to illustrate a frontrunner technology for personalized care of our wounded war fighters.

Our studies have enrolled wounded US service members who had sustained penetrating injuries to one or more extremities, who did not have confounding immunologic comorbid conditions, and who had been evacuated to the National Capital Area from Iraq and Afghanistan. Surgical debridement, pulse lavage wound irrigation and negative pressure wound therapy was repeated every 48–72 h until wound closure or coverage, according to current institutional standards of practice [20–22]. In general, these patients were young (average age of 23 years old, with a range between 18 and 37 years) and fit (with an average BMI under 25). Therefore, we were afforded a fairly homogeneous, athletic population within which to study the biological response to traumatic, acute injury and identify the early hallmarks of wound healing versus dehiscence.

Today's combat casualty care typically involves the aggressive surgical care of complex injuries caused by blasts and high-energy ballistics that inflict devastating trauma, often violating soft tissue, bone and neurovascular structures, especially in the extremities [5,23–27]. With the shift in modern war tactics towards an increased use of improvised explosive devices, high-powered munitions and close-quarter combat, war injuries and combat care increasingly differs from classical trauma management [4]. In our recent report, we monitored 52 extremity wounds in 33 patients with up to three wounds per patient. The primary clinical outcome measure was successful wound healing within 30 days of definitive closure or coverage with skin graft without necessitating a return to the operating room, spontaneous partial or complete wound disruption after primary closure, or over 90% skin-graft loss [20]. The wounds of that study covered an average surface area of 241 cm^2 (ranging between 25 and 1729 cm^2) and had an average volume of 369 cm^3 (ranging between 1.45 and 2119 cm^3). There was a mean patient injury severity score (ISS) of 20 (ranging between 8 and 45). These represent the typical modern combat wound that reaches tertiary care.

Treatment of war wounds requires a significant amount of time and resources directed toward either limb salvage or length preservation in amputations. In addition, up to 75% of these injuries arrive at tertiary care military medical facilities, such as the National Naval Medical Center (MD, USA) or Walter Reed Army Medical Center (DC, USA), colonized or infected with fastidious environmental bacteria such as *Acinetobacter baumannii* [28,29]. Local antibiotic delivery used in concert with irrigation, debridement and negative-pressure wound therapy have advanced the treatment of these injuries, but the basic surgical decision regarding the timing of wound closure or flap coverage of a wound remains subjective [21].

In the following sections, we describe our efforts to define the role of biomarkers associated with combat wound healing in this patient population. Our initial pilot study of procalcitonin (ProCT) and cytokine expression at the time of surgical wound closure is described, followed by a discussion of expanded studies investigating the longitudinal inflammatory response and, finally, the role of remodeling proteases in combat wounds.

Role of ProCT & cytokines at the time of wound closure

The utility of a well-defined biomarker profile of traumatic acute wounds that progress to successful healing after surgical closure was introduced by our pilot work, reported by Forsberg *et al.*, which investigated the correlation of ProCT and cytokine expression with war-wound dehiscence [21]. In that work, we posited that a diagnostic biomarker panel predictive of healing, as opposed to dehiscence, would be of great use in reducing the rate of wound failures caused by premature surgical closures. In addition, such a predictive diagnostic would reduce the number of surgical wound debridements a patient would undergo. In either case, a diagnostic biomarker panel that was predictive of healing would result in lower patient risk, reduced length of stay in hospital and a faster return to duty. In an effort to provide an objective measure of appropriate timing of traumatic wound surgical closure, the Forsberg *et al.* pilot study detected and quantified ProCT and cytokines in wound effluent as well as serum to preemptively differentiate the wounds that will heal uneventfully from those at risk of dehiscence.

Procalcitonin and C-reactive protein have been investigated as correlates of inflammation, complications and outcome in patients with multiple trauma [30,31]. Those studies were primarily concerned with determining the onset of septic complications to direct treatment. They reported that C-reactive protein had little to no diagnostic value as it was elevated owing to the initial trauma, demonstrated only a slow decline in concentration over the course of treatment, and did not indicate a significant difference between survival and nonsurvival groups. However, ProCT demonstrated a slight-to-moderate increase, which did correlate with severity of trauma and followed a relatively rapid return to baseline level in the absence of sepsis while remaining significantly elevated or further increasing at the moment of septic complications.

In agreement with these findings, and furthering their application to the evaluation of traumatic wound healing, Forsberg *et al.* first reported that elevated serum and effluent ProCT levels measured at the time of wound closure correlated with later wound dehiscence [21]. As reported, no wound failed with an effluent ProCT concentration of less than 220 pg/ml, IL-13 concentration greater than 12 pg/ml or Regulated on Activation, Normal T Expressed and Secreted (RANTES) protein concentration greater than 1000 pg/ml. It was also observed that there was a larger ratio of serum:effluent ProCT concentrations for wounds that healed (mean 114.2 pg/ml vs mean 36.01 pg/ml) compared with those that eventually dehisced (mean 560.8 pg/ml vs 338.6 pg/ml). These initial results highlighted the importance of both the local (effluent) and systemic (serum) response to trauma, and warranted an expanded study to further explore effluent cytokine and chemokine analysis as objective, decision-supportive biomarkers predictive of wound healing.

Inflammatory response to injury & combat wounds

In an expanded study, Hawksworth *et al.* demonstrated that kinetic expression of inflammatory cytokines and chemokines are objectively associated with acute war wound healing, and identified cytokine and chemokine protein, and gene transcripts with potential utility as biomarkers for predicting wound outcome (summarized in TABLE 1) [20]. This study involved analysis of the 52 wounds described previously in this article and reported that nine (17.3%) wounds across five patients dehisced; three of these patients dehisced all of their wounds. Significant clinical determinants for wound dehiscence included elevated ISS ($p = 0.03$), larger wound surface area ($p = 0.005$) and volume ($p = 0.004$), and associated vascular injury ($p = 0.002$) [20]. While the clinical association between systemic illness and impaired wound healing is established [32,33], Hawksworth *et al.* reported a panel of markers that seemingly report some of the biological mechanisms responsible for healing traumatic injuries.

Using multiplexed protein quantitation of serum and effluent combined with quantitative real-time PCR of wound biopsies, significant and predictive differences between wounds that progress to healing and those that fail within 30 days of surgical closure are described. With an analysis across serial wound debridements, receiver operating characteristic curves for serum IL-6, IL-8,

macrophage inflammatory protein (MIP)-1α, effluent IFN-γ inducible protein 10 and IL-6 protein biomarkers were each statistically predictive of wound dehiscence with favorable receiver operating characteristic curves (area under the curve of 0.805, 0.755, 0.695, 0.660 and 0.632 respectively; $p < 0.05$). The tissue gene transcripts *MCP-1*, *IL-1α*, *TNF-α*, *IL-8*, *MIP-1α*, *GM-CSF*, *IL-1β* and *-6* were also individually statistically predictive of wound dehiscence (0.845, 0.845, 0.845, 0.832, 0.755, 0.744, 0.729 and 0.662, respectively; $p < 0.05$) [20].

Wound remodeling proteases are associated with wound failure

The equilibrium of inflammatory mediators is crucial for the progression of acute wound healing. In the study by Hawksworth *et al.*, a state of systemic inflammatory dysregulation was supported by consistently elevated serum protein IL-6, -8 and MIP-1α in patients with wound dehiscence. Such a prolonged inflammatory response involving an important mediator of the acute-phase response, and potent chemoattractants of macrophages and granulocytes is consistent with a wounds delayed advance into the fibroproliferative phase, in which collagen deposition and rapid gains in wound-breaking strength typically occur [20,34].

In addition, MMPs play crucial roles in the inflammation, proliferation and maturation phases by performing several functions related to inflammatory signaling (establishing chemotactic gradients that direct immune cell migration) and wound remodeling (processing collagens and elastin) [35–37]. Elevated levels of the proteases MMP-2 (gelatinase) and MMP-3 (stromelysin) and low levels of their respective inhibitors have been measured in wound effluent of chronic pressure ulcers treated with negative-pressure wound therapy [38]. Furthermore, disproportionate expression of MMPs and their inhibitors has been proposed as a cause of wound chronicity [37,39–41]. By analyzing concentrations of MMP-2, -3, -7 (matrilysin), -9 (gelatinase) and -13 (collagenase) in patent serum and traumatic extremity wound effluent throughout the treatment process, the work presented by Hawksworth *et al.* was extended to include these representative MMPs as potential objective markers of healing and, furthermore, as indicators of timing for successful surgical wound closure and avoidance of dehiscence in traumatic acute wounds [22].

In the subset of wounds analyzed (38 wounds in 25 patients; up to three wounds per patient), MMP expression was associated with impaired wound healing, which was defined as wounds that dehisced or required delayed definitive wound closure of 21 days or more after injury (two standard deviations outside of the mean normal wound closure time period of 10 days) [22]. It was found that proinflammatory MMP-2 and -7 levels, sampled at each surgical debridement, were statistically higher in patients that demonstrated impaired wound healing as opposed to patients with wounds that healed normally ($p < 0.001$) (see Table 1). In addition, serum MMP-7 differed significantly when grouped by vascular injury or ISS [22]. Patients with impaired wound healing expressed statistically lower levels of effluent MMP-3, a wound resolution MMP, throughout the debridement and healing process when compared with patients with wounds that healed normally ($p < 0.001$). Finally, receiver operating characteristic curve analysis revealed that serum MMP-2 and -7, and

Table 1. Candidate biomarkers for combat wound healing.

Biomarker	AUC
Inflammatory cytokines†	
Tissue *MCP-1*	0.845
Tissue *IL-1α*	0.845
Tissue *TNF*	0.845
Tissue *IL-8*	0.832
Serum IL-6	0.805
Serum IL-8	0.755
Tissue *MIP-1α*	0.755
Tissue *GM-CSF*	0.744
Tissue *IL-1β*	0.729
Serum MIP-1α	0.695
Tissue *IL-6*	0.662
Effluent IP-10	0.660
Effluent IL-6	0.632
Effluent IL-2	0.612
Remodeling proteases‡	
Effluent MMP-3	0.805
Serum MMP-7	0.783
Serum MMP-2	0.744
Effluent MMP-9	0.661
Serum MMP-9	0.655
Effluent MMP-13	0.644
Effluent MMP-7	0.632

Inflammatory cytokines and remodeling proteases associated with combat wound dehiscence. Tissue refers to transcript analysis for wound biopsies, serum refers to protein analysis of serum samples and effluent refers to protein analysis of effluent from negative-pressure wound therapy.
†Data taken from [23].
‡Data taken from [25].
AUC: Area under the curve; IP: Interferon-inducible protein; MIP: Macrophage inflammatory protein; MMP: Matrix metalloproteinase.

effluent MMP-3 were each statistically predictive of wound healing throughout the initial three and final debridements prior to surgical wound closure (area under the curve of 0.744, 0.783 and 0.805, respectively; $p < 0.001$) [22].

In Utz *et al.*, the interplay between markers of prolonged systemic response to injury and local mediators of the healing process is further illustrated. It is pointed out that while MMP-2's collagen and basement membrane cleaving activity, as well as immune cell recruitment, are important to this process, excessive action has clear detrimental consequences for wound healing. It is further elaborated that the constitutively high expression of serum MMP-7 (involved in extracellular matrix processing, re-epithelialization and neutrophil migration) in impaired healing wounds is consistent with an exaggerated inflammatory response and a component of 'stalling' the healing process [22]. Admittedly, it was not clear whether the elevated MMP-7 was directly linked to impaired wound healing or if it was instead reporting the associated vascular injury. Nonetheless, it presents as a potential classifier of normal healing wounds versus those that will require additional medical attention. Finally, the investigation also identified MMP-3, an important component of wound remodeling and resolution, as elevated in wounds with normal healing rates versus reduced levels in impaired wounds [22].

Using a biomarker panel for a bench-to-bedside approach

Whereas the previously reviewed reports identified biomarkers that were individually predictive of outcome, we would be remiss not to acknowledge that statistically significant differences in quantifiable biomarkers do not necessarily equate to clinical practicality or utility. As many of the analytes investigated for use as indices of wound healing or infection are components of overlapping biological processes, it can become exceedingly difficult to adequately describe the patient (or wound) for whom a single-analyte diagnostic would function properly. Instead, systems-biology approaches may be more appropriate in translating biomarkers from bench to bedside. One method to analyze data in a systems-based approach is by using advanced machine-learning algorithms.

Preliminary work within our research group has demonstrated that machine-learned Bayesian belief networks, which identify conditional dependence between variables and present this structure in a graphical format, to predict acute war wound outcome from an integrated gene-expression panel could further enhance wound classification [Brown T, Eberhardt J, Elster E, Unpublished Data]. Hawksworth *et al.* concluded that the cytokine and chemokine protein and gene transcript expression patterns demonstrated a condition of inflammatory dysregulation associated with war wound failure [20]. To move those findings closer to the bedside, we hypothesized that a combined molecular biomarker panel may predict combat wound-healing outcome when considered together in a single algorithm classifying the multivariate dependence relationships. Full Bayesian analysis of cytokine and chemokine expression allowed for effective predictive modeling of the occurrence of wound healing. In addition, the coordinated dependence relationships of our Bayesian model corroborate the univariant analysis with the addition of an evidence-based risk of wound dehiscence prediction [Brown T, Eberhardt J, Elster E, Unpublished Data].

Preliminary modeling included the protein quantitation for each wound at first, second, third and final surgical wound debridement as separate variables, which were categorized by distribution into three equal probability density groups. Models were derived in stepwise iterations until the optimal network was identified, as determined by cross-validation and qualitative assessment against clinical experience and the literature. To evaluate robustness of the Bayesian models, each patient was serially excluded from the dataset in a leave-one-out cross-validation [42].

Multivariate models classifying healing or microbial infection in complex traumatic wounds will need to be validated in randomized clinical trials in which model-based patient care is performed and the response of the model to associated changes in care (e.g., earlier surgical wound closures or administration of antibiotics) can be evaluated. Owing to the complexity of these types of injuries compounded by a prevalence of microbial infection, we believe that advances in the field of wounded wartime care will come from such integrated panels of patient-specific markers of wound healing.

Conclusion

The assessment of the suitability for combat wound closure is evolving from subjective clinical observation and assessment of microbial status using early 20th century culture methods to an understanding of the inflammatory environment in which wound healing occurs. This shift has occurred with a change in combat-related

morbidity towards larger extremity wounds in patients that are more acutely ill. While our group has begun to describe the relationship between the inflammatory response to trauma and the ability to predict wound healing in combat casualties, it is not to be assumed unique to this patient population as others have demonstrated a significant link between systemic inflammation and serum cytokines with clinical outcome [43]. Many of the cytokines seen in such studies of civilian trauma patients overlap with ones measured in combat-wounded patients, suggesting the utility of this approach beyond the military-specific injury.

The ability to assess a patient's inflammatory response, both systemically and locally, and develop decision-support tools based on these biomarkers introduces the use of personalized medicine in the treatment of trauma patients based on the individual's biology rather than the classical prescription that is 'best for most' or population-based care. While still a developing approach to diagnosis and treatment of illness and disease, personalized medicine is becoming a pivotal mechanism by which doctors treat patients. In the treatment of disease, personalized medicine uses the genetic make-up of an individual, both static (as in their DNA sequence) and dynamic (as in DNA modifications or expressed RNA and proteins), to determine which treatment approach will be most effective for an illness. As we have presented here, measurement of an individual's cytokine, chemokine, MMPs, and ProCT expression in response to injury may, in the near future, direct traumatic wound-treatment decisions. Therefore, by using a personalized medicine approach, clinicians can predetermine the most effective treatment for the most beneficial outcome per patient instead of treatments based on benefits for a generalized population.

We concede that the current method of determining wound closure is often successful. That is, an experienced surgeon can observe a wound and objectively evaluate characteristics such as size, color and effluent to subjectively predict whether more debridements are necessary or if the wound will close successfully. Using biomarkers as a predictive mechanism, however, is expected to not only allow these decisions to be made by less experienced surgeons, but to both increase the success rate of wound closures and reduce the number of operating room visits per wound prior to deciding to perform such surgical closures. These early steps, with regards to wound closure, will allow for the assessment of several other aspects of trauma care that are mentioned later.

Future perspective

An objective decision on wound closure, based on changes in an individual's own biology, provides a clear benefit for the treatment of trauma patients in the near term. But this method could prove successful for other indications as well. As wound failure has been associated with other patient outcomes, applying this data to other clinical scenarios could guide therapeutics. Pneumonia, for example, is an outcome commonly associated with patients with traumatic wounds. If a biomarker panel of protein and gene expression can be linked with the development of pneumonia, doctors will be empowered for early intervention by a prediction of a patient's risk for contracting the disease. Other possible disease processes that can be addressed in a similar fashion include traumatic brain injury, heterotopic ossifications and infectious complications.

Ideally, this predictive mechanism will be applicable for multiple clinical indicators within any single patient. With multiplexed biomarker panels associated with clinical outcomes, clinicians could assay multiple targets with a single patient sample. Not only will this reduce the burden on the patient by requiring fewer sample collections, but costs will also be decreased by requiring fewer tests.

The ability to scan for multiple clinical problems could also lead to improved care of wounded patients. By predicting wound outcome, some patients may experience a reduced number of operative procedures (irrigation and debridements) prior to wound closure and a shorter hospital stay. Conversely, predicting when a wound is not ready for closure will allow hospital staff to anticipate and prepare for a longer stay in the intensive care unit for that patient. Similarly, by predicting other outcomes in addition to successful wound closure, doctors can anticipate whether specific clinical complications will influence a patient's treatment plan.

In wars of the future, patients will experience improved and accelerated care. Wounded patients will enter any hospital and clinicians will be able to make a series of predictions using only a tissue, serum or effluent sample. Analyzing a series of biomarker panels in a single assay will allow doctors to objectively decide to close a wound in one patient or to use systemic therapy to prevent secondary complications for another patient. These therapeutic advances will provide for improved patient care and quicker return to duty for our injured fighters. As with all previous conflicts, the lessons learned from

current and future wars will provide a template for the treatment of civilian injuries and the next conflict.

Disclosure

The views expressed in this article are those of the authors and do not reflect the official policy or the position of the Department of the Navy, Department of the Army, the Department of Defense or the US Government. This work was prepared as part of their official duties.

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Executive summary

- As the mortality rate in combat-wounded patients has decreased, the severity of injuries presented to military-care facilities has increased in current conflicts.
- Past decisions regarding combat wound closure were made with a subjective assessment of the wound coupled with an assessment of its bacterial burden.
- Wound failure in combat-wounded patients is dependent on the local and systemic inflammatory response to injury coupled with the bacterial burden.
- Assessment of this response using serum and wound-effluent biomarkers may allow for the development of predictive assays for the timing of wound closure.
- Development of personalized medicine for combat-injured patients has utility beyond wound care and will be deployable to civilian hospitals as well as in future conflicts.

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